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EXAMINER	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/600,201	SLEPNEV, VLADIMIR I.	
	Examiner	Art Unit	
	ANGELA BERTAGNA	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *18 July 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *18,19,21-35,37-49,62,63 and 65-75* is/are pending in the application.

4a) Of the above claim(s) *74 and 75* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *18,19,21-35,37-49,62,63 and 65-73* is/are rejected.

7)☒ Claim(s) *38 and 66* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *3/24/08; 6/18/08; 9/29/08*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on July 18, 2008 has been entered.

Status

2. Claims 18, 19, 21-35, 37-49, 62, 63, and 65-75 are currently pending. In the response, Applicant amended claims 18, 21, 22, 26, 34, 37, 42, 62, 65, and 69 and canceled claims 1-17, 20, 36, 50-61, and 64. Claims 74 and 75 are withdrawn from consideration as being drawn to a non-elected invention.

Applicant's amendments to the claims have overcome the rejection of claims 1, 2, 5, 10, 12, 18, 19, 22, 27, 34, 35, 38, and 43 under 35 U.S.C. 102(b) as being anticipated by Myakishev, and therefore, it has been withdrawn.

Applicant's arguments that remain applicable to the new grounds of rejection presented below have been fully considered, but they were not persuasive for the reasons set forth in the "Response to Arguments" section.

Information Disclosure Statement

3. Applicant's submission of an Information Disclosure Statement on September 29, 2008, June 18, 2008, and March 24, 2008 is acknowledged. Signed copies are enclosed.

It is noted that the information disclosure statement filed on June 18, 2008 fails to comply with 37 CFR 1.98(a)(2), which requires a legible copy of each cited foreign patent document; each non-patent literature publication or that portion which caused it to be listed; and all other information or that portion which caused it to be listed. It has been placed in the application file, but the information referred to therein has not been considered. More specifically, a copy of WO 04/048528 has not been provided, and accordingly, this reference has not been considered.

Claim Objections

4. Claims 38 and 66 are objected to because of the following informalities: These claims contain a typographical error. Replacing "comprising" in line 1 with "comprises" is suggested.

Appropriate correction is required.

Claim Rejections - 35 USC § 112, 2nd paragraph

5. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 43, 62, 63, and 65-73 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 43 is indefinite, because it recites the limitation "said tag sequence" in line 1. There is insufficient antecedent basis for this limitation in the claim. There is sufficient antecedent basis for "said first tag sequence", "said second tag sequence", or "said first and second tag sequences". The lack of antecedent basis in claim 43 causes uncertainty as to whether the recited length limitation applies to only the first tag sequence, only the second tag sequence, or to the first and second tag sequences.

Claims 62, 63, and 65-73 are indefinite, because independent claim 62 recites the limitation "said set of second oligonucleotide primers" in step VII. There is insufficient basis for this limitation in the claim.

Claim 73 is further indefinite, because it recites the limitation "said set of distinguishably labeled downstream amplification primers" in lines 1-2. There is insufficient antecedent basis for this limitation in the claim.

Claim Rejections - 35 USC § 103

6. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

7. Claims 18, 19, 21, 22, 26, 27, 34, 35, 37, 38, 42, and 43 are rejected under 35 U.S.C. 103(a) as being unpatentable over Myakishev et al. (Genome Research (January 2001) 11: 163-169; cited previously) in view of Piggee et al. (Journal of Chromatography A (1997) 781(1-2): 367-75; cited previously).

These claims are drawn to an amplification-based method of determining the identities of the nucleotides at a set of known polymorphic sites.

Regarding claim 18, Myakishev teaches a method of determining, for a given nucleic acid sample, the identities of the nucleotides at a set of known polymorphic sites to be interrogated (see Figure 2 and pages 163-165, where nine SNPs were tested), comprising:

(a) subjecting to an amplification regimen, a population of primer extension products generated from a nucleic acid sample (see Figure 2, top panel, where extension with the tailed primers occurs in round 1 of the amplification to generate the population of primer extension products; see also page 164, column 2, steps 3-4 shown in Figure 2 and page 164, column 2, where amplification of these primer extension products is taught),

wherein each primer extension product comprises a member of a set of tag sequences, that specifically corresponds to the presence of one specific nucleotide at a known polymorphic site (see page 164, column 1, where Myakishev teaches that the allele-specific primers contain different 5' tags), and

wherein the amplification regimen is performed using one upstream amplification primer for each sequence comprising a known polymorphic site to be interrogated and a set of distinguishably labeled downstream amplification primers (see page 163 and Figure 2, where the reverse primer is the upstream primer and the ET primers are the set of distinguishably labeled downstream primers), and

wherein each member of the set of downstream amplification primers comprises a tag sequence comprised by a member of the population of primer extension products and a

distinguishable label that specifically corresponds to the presence of a specific nucleotide at the polymorphic site (see Figures 1 & 2), and

wherein the upstream amplification primers are selected such that each polymorphic site of the set of known polymorphic sites to be interrogated corresponds to a distinctly sized amplification product (see the table below, where the upstream (reverse) primers inherently hybridize at distinct distances 5' of the polymorphic sites present in the genomic DNA template used in the method of Myakishev to produce distinctly sized amplicons), and

(b) detecting the incorporation of a distinguishable label in distinctly sized amplification products, thereby determining the identity of the nucleotide at each the polymorphic site (see Figure 2 and also the Methods section at page 168, where fluorescence is detected).

Regarding claim 34, Myakishev teaches a method of determining, for a given nucleic acid sample, the identities of the nucleotides at a set of known polymorphic sites to be interrogated (see Figure 2 and pages 163-165, where nine SNPs were tested), comprising:

a) subjecting to an amplification regimen, a population of primer extension products generated from a nucleic acid sample (see Figure 2 and page 164, column 2), wherein each primer extension product comprises a first tag sequence (generated by extension of the reverse primer) or its complement and a member of a set of second tag sequences or its complement (the 21 nt tail added in step 1 of the reaction), and

wherein the presence of the second tag sequence or its complement specifically corresponds to the presence of one specific nucleotide at a known polymorphic site (page 164, column 1), and

wherein for each polymorphic site in the set of polymorphic sites, the first tag sequence is located at a distinct distance 5' of the polymorphic site, relative to the distance of the first tag sequence from a polymorphic site on molecules in the sample containing other polymorphic sites (see the table below, where the reverse primers inherently hybridize at distinct distances 5' of the polymorphic sites present in the genomic DNA template used in the method of Myakishev), and

wherein the amplification regimen is performed using an upstream amplification primer comprising the first tag sequence (the reverse primer, page 163), and a set of distinguishably labeled downstream amplification primers (the ET primers), and

wherein each member of the set of downstream amplification primers comprises a tag sequence comprised by a member of the population of primer extension products (*i.e.* the tail sequence; see Figure 1) and a distinguishable label that specifically corresponds to the presence of a specific nucleotide at said polymorphic site (see Figures 1 & 2),

and wherein the upstream amplification primers are selected such that each polymorphic site of the set of known polymorphic sites to be interrogated corresponds to a distinctly sized amplification product (see the table below, which indicates that the upstream primers used in the method of Myakishev produce distinctly sized amplicons), and

b) detecting the incorporation of a distinguishable label in distinctly sized amplification products, thereby determining the identity of the nucleotide at each polymorphic site (page 168, column 1).

SNP name	Amplicon size (nt)
CCK	79
CYP17	131
DRD1	150
DRD2	87
HTR1B	80
HTR2A	93
HTR2C	50
MAOA	97

Regarding claims 19 and 35, a fluorescent label is used in the method of Myakishev (see Figure 2).

Regarding claims 22 and 38, the amplification regimen conducted in the method of Myakishev comprises at least two amplification reaction cycles, with each cycle comprising the steps of: 1) nucleic acid strand separation; 2) oligonucleotide primer annealing; and 3) polymerase-mediated extension of annealed primers (see Figure 2 and page 164; see also pages 167-168 for the reaction conditions).

Regarding claims 26 and 42, Myakishev teaches a thermal cycling device with a fluorescence detection system (page 168). It is noted that the specification does not require the elements of the modular apparatus of claims 26 and 42 (*i.e.* a thermal cycling device, a sampling device, a capillary electrophoresis device and a fluorescence detector) to be physically connected.

Regarding claims 27 and 43, Myakishev teaches that the tag sequence is 21 nucleotides in length (see page 164, column 1).

Myakishev teaches fluorescence detection of the distinctly sized amplification products rather than separation by size and/or by charge, and specifically by capillary electrophoresis, as required by claims 18, 21, 23-26, 34, 37, and 39-42.

Piggee teaches a method for genotyping single nucleotide polymorphisms (see abstract).

Regarding claims 18, 21, 34, and 37, the method of Piggee comprises single nucleotide primer extension and capillary electrophoresis separation with laser-inducted fluorescence detection (see abstract and pages 368-370).

Regarding claims 26 and 42, Piggee teaches a capillary electrophoresis device with a sampling device, and a fluorescence detection system (pages 368-369).

Piggee teaches that capillary electrophoresis method is fast, avoids radioactive labels, and has a high automation potential (see abstract and page 375). Although Piggee used capillary electrophoresis to detect point mutations analyzed by single nucleotide primer extension reactions, it is expressly stated in the reference that the method would also be applicable to other methods of mutation detection including competitive priming with end mismatch, etc (page 375). Piggee also noted that the capillary electrophoresis method facilitated multiplexing through the use of different length primers (page 375).

It would have been *prima facie* obvious for one of ordinary skill in the art at the time of invention to utilize capillary electrophoresis as an additional or alternative method of detection when practicing the method taught by Myakishev. As discussed above, Piggee expressly taught the advantages of a capillary electrophoresis detection system, namely, rapid analysis with a high

potential for automation and multiplexing (see abstract and page 375). An ordinary artisan would have been motivated by these teachings of Piggee to additionally or alternatively analyze amplification products generated by the method of Myakishev using capillary electrophoresis as taught by Piggee in order to more rapidly and automatically screen a large number of samples with the additional control of length-based detection. Thus, the methods of claims 18, 19, 21, 22, 26, 27, 34, 35, 37, 38, 42, and 43 are *prima facie* obvious over Myakishev in view of Piggee.

8. Claims 23-25 and 39-41 are rejected under 35 U.S.C. 103(a) as being unpatentable over Myakishev et al. (Genome Research (January 2001) 11: 163-169; cited previously) in view of Piggee et al. (Journal of Chromatography A (1997) 781(1-2): 367-75; cited previously) and further in view of Woolley et al. (Analytical Chemistry (1996) 68: 4081-4086; cited previously).

The combined teachings of Myakishev and Piggee result in the methods of claims 18, 19, 21, 22, 26, 27, 34, 35, 37, 38, 42, and 43, as discussed above.

Regarding claims 25 and 41, Piggee teaches a method of detecting point mutations using capillary electrophoresis and laser-induced fluorescence detection (abstract and pages 368-370).

Neither Myakishev nor Piggee teaches analyzing an aliquot of the PCR after each reaction cycle by capillary electrophoresis as required by claims 23, 24, 39, and 40.

Woolley teaches a method for conducting PCR amplification and capillary electrophoresis in a microfabricated DNA analysis device (see abstract and pages 4082-4084). Regarding claims 23-25 and 39-41, Woolley teaches taking an aliquot after 15, 20, 25, and 30 cycles of a PCR reaction and analyzing the amount of accumulating product by capillary electrophoresis with fluorescence detection to obtain real-time monitoring of product

accumulation (see page 4085 and also Figure 5). Woolley also teaches that the disclosed integrated PCR-CE microdevices permit very rapid thermal cycling and electrophoretic separation, are suitable for use in virtually any other amplification method, permit less expensive real-time monitoring of product accumulation, and eliminate the need for sample handling between the amplification and capillary electrophoresis steps, thereby simplifying the process, eliminating pipetting errors, and reducing contamination opportunities (page 4086, column 2).

It would have been *prima facie* obvious for one of ordinary skill in the art at the time of invention to utilize the integrated PCR-CE microdevice taught by Woolley when practicing the method resulting from the combined teachings of Myakishev and Piggee. An ordinary artisan would have been motivated to do so, since Woolley taught that the integrated PCR-CE microdevices permit very rapid thermal cycling and electrophoretic separation, are suitable for use in virtually any other amplification method, permit less expensive real-time monitoring of product accumulation, and eliminate the need for sample handling between the amplification and capillary electrophoresis steps, thereby simplifying the process, eliminating pipetting errors, and reducing contamination opportunities (page 4086, column 2). An ordinary artisan also would have been motivated to monitor aliquots of the amplification reaction mixture in order to monitor product accumulation in real-time as suggested by Woolley (see page 4085 & Figure 5). Finally, regarding claims 24 and 40, an ordinary artisan would have recognized from the teachings of Woolley that the number of aliquots analyzed during an amplification reaction was a results-effective variable that should be optimized by routine experimentation. As noted in MPEP 2144.05, it is *prima facie* obvious to optimize results-effective variables using routine experimentation in the absence of unexpected results. In this case, an ordinary artisan would

have recognized that analyzing more aliquots (*e.g.* after each reaction cycle) would have improved the method resulting from the combined teachings of Myakishev, Piggee, and Woolley by providing more data points for analysis and a clearer picture of the exponential and plateau phases of the amplification process. Thus, the methods of claims 23-25 and 39-41 are *prima facie* obvious in view of the combined teachings of Myakishev, Piggee, and Woolley.

9. Claims 28-33, 44-49, 62, 63, 65, 66, and 69-73 are rejected under 35 U.S.C. 103(a) as being unpatentable over Myakishev et al. (Genome Research (January 2001) 11: 163-169; cited previously) in view of Piggee et al. (Journal of Chromatography A (1997) 781(1-2): 367-75; cited previously) and further in view of Nolan et al. (US 6,287,766 B1; cited previously).

The combined teachings of Myakishev and Piggee result in the method of claims 18, 19, 22, 26, 27, 34, 35, 37, 38, 42, and 43, as discussed above.

Regarding claim 62, Myakishev teaches a method of determining the identity of a single nucleotide at a known polymorphic site (see Figure 2 and pages 163-165), comprising:

I) providing a nucleic acid sample comprising the polymorphic site (see Figure 2 and page 168)

II) separating the strands of the nucleic acid sample and re-annealing in the presence of:

(a) a first oligonucleotide primer comprising a 3' region that hybridizes to a sequence at a known distance downstream of the known polymorphic site, wherein the first oligonucleotide primer comprises a first sequence tag located 5' of the 3' region (see Figure 2, where the reverse primer is taught. It is noted that since the specification

defines a tag as any nucleic acid sequence in a primer, any dinucleotide of the reverse primer is a tag)

(b) a set of second oligonucleotide primers (the allele-specific primers of Figure 2), wherein each member of the set comprises: (i) a region that hybridizes 5' of and adjacent to the polymorphic site, (ii) a variable 3' terminal nucleotide, wherein, when the member is hybridized to the known sequence, the 3' terminal nucleotide is opposite the polymorphic site, and wherein, if and only if the 3' terminal nucleotide is complementary to the nucleotide at the polymorphic site, the 3' terminal nucleotide base pairs with the nucleotide at the polymorphic site (see Figure 2 and page 164), and (iii) a tag sequence that corresponds to the variable 3'-terminal nucleotide of (ii) with the tag sequence located 5' of the region of (i) on the member (see the tail sequence in Figure 2)

III) contacting the annealed oligonucleotides resulting from step (II) with a nucleic acid polymerase under conditions that permit the extension of an annealed oligonucleotide such that extension products are generated, wherein the primer extension product from the first oligonucleotide primer, when separated from its complement, can serve as a template for the synthesis of the extension product of a member of the set of second oligonucleotide primers (see Figure 2), and vice versa

IV) repeating the strand separating and contacting steps (*i.e.* steps (II) and (III)) twice, such that a population of nucleic acid molecules is generated that comprises both a sequence identical to or complementary to the first oligonucleotide and a sequence identical to or complementary to one of the members of the second set of oligonucleotides (see Figure 2 and page 164)

V) subjecting the population of nucleic acid molecules to an amplification regimen, wherein the amplification regimen is performed using an upstream amplification primer comprising the first sequence tag comprised by the first oligonucleotide primer (*i.e.* the reverse primer, as discussed above), and a set of downstream amplification primers, each member of the set of downstream amplification primers comprising a tag comprised by a member of the set of second oligonucleotide primers and a distinguishable label (*i.e.* the energy transfer primers that comprise the tail sequence; see Figure 1)

VIII) detecting the incorporation of at least one distinguishable label, thereby determining the identity of the nucleotide at the known polymorphic site (see Figure 2 and page 168).

Further regarding claim 62, it is noted that the designations "upstream" and "downstream" are arbitrary designations depending on the choice of the reference strand, and therefore, the primers of Myakishev meet the claimed limitations. Also, the primers used by Myakishev produce distinctly sized amplification products as noted above with regard to claims 18 and 34.

Regarding claim 63, the method of Myakishev uses fluorescent labels (Figure 2).

Regarding claim 66, the amplification regimen conducted by Myakishev comprises at least two amplification reaction cycles, wherein each cycle comprises the steps of: 1) nucleic acid strand separation; 2) oligonucleotide primer annealing; and 3) polymerase extension of annealed primers (Figure 2 and page 164; see also pages 167-168 for the reaction conditions).

Regarding claim 69, Myakishev teaches a thermal cycling device with a fluorescence detection system (page 168). It is noted that the specification does not require the elements of

claim 69 (*i.e.* a thermal cycling device, a sampling device, a capillary electrophoresis device and a fluorescence detector) to be physically connected.

Regarding claim 70, Myakishev teaches that the tail portions of the allele-specific primers are 21 nucleotides in length (page 164, column 1). Also, since the entire 20 nt reverse primer comprises a tag, both the first and second tags comprise 15 to 40 nucleotides.

Regarding claims 71 and 72, the 20 nt reverse primer hybridizes with perfect complementarity to the target downstream of the polymorphic site, and the allele-specific primers have a 22 nt target-specific portion that hybridizes upstream of the polymorphic site (see Table 1).

Myakishev does not teach exonuclease digestion to remove unextended primers as required by claims 29-33, 45-49, and 62. Also, regarding claim 62, Myakishev does not teach the use of multiple first primers or separating the nucleic acid molecules by size and/or charge, and specifically by capillary electrophoresis. Finally, regarding claims 28, 44, and 73, Myakishev teaches using a set of downstream primers comprising two different nucleotide bases at the 3'end, for example, A and G (see Figure 2), but does not teach using a set of downstream primers consisting of four primers, each with a different 3'-terminal nucleotide.

Piggee teaches a method for genotyping single nucleotide polymorphisms (see abstract).

Regarding claim 62 and 65, the method of Piggee comprises single nucleotide primer extension and capillary electrophoresis separation with laser-inducted fluorescence detection (see abstract and pages 368-370).

Regarding claim 69, Piggee teaches a capillary electrophoresis device with a sampling device, and a fluorescence detection system (pages 368-369).

Piggee teaches that capillary electrophoresis method is fast, avoids radioactive labels, and has a high automation potential (see abstract and page 375). Although Piggee used capillary electrophoresis to detect point mutations analyzed by single nucleotide primer extension reactions, it is expressly stated in the reference that the method would also be applicable to other methods of mutation detection including competitive priming with end mismatch, etc (page 375). Piggee also noted that the capillary electrophoresis method facilitated multiplexing through the use of different length primers (page 375).

Nolan teaches a method of identifying polymorphisms using flow cytometry.

Regarding claims 28, 44, and 73, the method taught by Nolan in Example 5 (column 7, lines 1-63) comprises an oligonucleotide ligation assay (OLA) followed by PCR amplification with an upstream primer and a set of downstream primers that may be fluorescently labeled (column 7, lines 11-50). Nolan teaches that the downstream primers each have a different 3'terminal nucleotide (column 7, lines 27-30) in order to identify the polymorphism in a single reaction.

Regarding claims 29-33, 45-49, and 62, Nolan teaches an embodiment wherein unincorporated primers from an initial amplification reaction are degraded using the heat labile Exonuclease I followed by polymerase extension, where the initial denaturation step destroys the activity of the exonuclease (see for example, column 5, line 60 – column 6, line 20). Nolan also teaches multiplex detection of mutations using multiple sets of primers (column 7, lines 60-63).

It would have been *prima facie* obvious for one of ordinary skill in the art at the time of invention to utilize capillary electrophoresis as an additional or alternative method of detection when practicing the method taught by Myakishev. As discussed above, Piggee expressly taught

the advantages of a capillary electrophoresis detection system, namely, rapid analysis with a high potential for automation and multiplexing (see abstract and page 375). An ordinary artisan would have been motivated by these teachings of Piggee to additionally or alternatively analyze amplification products generated by the method of Myakishev using capillary electrophoresis as taught by Piggee in order to more rapidly and automatically screen a large number of samples with the additional control of length-based detection.

It also would have been *prima facie* obvious for one of ordinary skill in the art at the time of invention to apply the teachings of Nolan to the method of Myakishev. An ordinary artisan would have recognized from the teachings of Nolan that not all polymorphic sites may be genotyped using only two allele-specific downstream primers, and therefore, would have been motivated to utilize a subset of four downstream primers each with a different 3'terminus, in order to accurately type these polymorphisms. Also, an ordinary artisan would have been motivated by the teachings of Nolan to perform a multiplexed analysis, utilizing multiple primer pairs in order to more rapidly and reproducibly genotype several sites simultaneously. Furthermore, an ordinary artisan would have been motivated by the teachings of Nolan to incorporate an exonuclease digestion step following the initial primer extension reaction with the allele-specific primers. Although Myakishev attempted to avoid mispriming by careful design of the allele-specific and energy-transfer primers (see page 164, column 1), incorporation of the exonuclease digestion step taught by Nolan following the initial extension would have eliminated the possibility of such mispriming events, and thereby improved the accuracy of the method. Thus, the methods of claims 28-33, 44-49, 62, 63, 65, 66, 69, and 73 are prima facie obvious in view of the combined teachings of Myakishev, Piggee, and Nolan.

10. Claims 67 and 68 are rejected under 35 U.S.C. 103(a) as being unpatentable over Myakishev et al. (Genome Research (January 2001) 11: 163-169; cited previously) in view of Piggee et al. (Journal of Chromatography A (1997) 781(1-2): 367-75; cited previously) and further in view of Nolan et al. (US 6,287,766 B1; cited previously) and further in view of Woolley et al. (Analytical Chemistry (1996) 68: 4081-4086; cited previously).

The combined teachings of Myakishev, Piggee, and Nolan result in the methods of claims 28-33, 44-49, 62, 63, 65, 66, and 69-73, as discussed above.

Regarding claims 67 and 68, Piggee teaches a method of detecting point mutations using capillary electrophoresis and laser-induced fluorescence detection (abstract and pages 368-370).

Myakishev, Piggee, and Nolan do not teach analyzing an aliquot of the PCR after each reaction cycle by capillary electrophoresis as required by claims 67 and 68.

Woolley teaches a method for conducting PCR amplification and capillary electrophoresis in a microfabricated DNA analysis device (see abstract and pages 4082-4084). Regarding claims 67 and 68, Woolley teaches taking an aliquot after 15, 20, 25, and 30 cycles of a PCR reaction and analyzing the amount of accumulating product by capillary electrophoresis with fluorescence detection to obtain real-time monitoring of product accumulation (see page 4085 and also Figure 5). Woolley also teaches that the disclosed integrated PCR-CE microdevices permit very rapid thermal cycling and electrophoretic separation, are suitable for use in virtually any other amplification method, permit less expensive real-time monitoring of product accumulation, and eliminate the need for sample handling between the amplification and capillary electrophoresis steps, thereby simplifying the process, eliminating pipetting errors, and reducing contamination opportunities (page 4086, column 2).

It would have been *prima facie* obvious for one of ordinary skill in the art at the time of invention to utilize the integrated PCR-CE microdevice taught by Woolley when practicing the method resulting from the combined teachings of Myakishev, Piggee, and Nolan. An ordinary artisan would have been motivated to do so, since Woolley taught that the integrated PCR-CE microdevices permit very rapid thermal cycling and electrophoretic separation, are suitable for use in virtually any other amplification method, permit less expensive real-time monitoring of product accumulation, and eliminate the need for sample handling between the amplification and capillary electrophoresis steps, thereby simplifying the process, eliminating pipetting errors, and reducing contamination opportunities (page 4086, column 2). An ordinary artisan also would have been motivated to monitor aliquots of the amplification reaction mixture in order to monitor product accumulation in real-time as suggested by Woolley (see page 4085 & Figure 5). Finally, regarding claim 68, an ordinary artisan would have recognized from the teachings of Woolley that the number of aliquots analyzed during an amplification reaction was a results-effective variable that should be optimized by routine experimentation. As noted in MPEP 2144.05, it is *prima facie* obvious to optimize results-effective variables using routine experimentation in the absence of unexpected results. In this case, an ordinary artisan would have recognized that analyzing more aliquots (*e.g.* after each reaction cycle) would have improved the method resulting from the combined teachings of Myakishev, Piggee, Nolan, and Woolley by providing more data points for analysis and a clearer picture of the exponential and plateau phases of the amplification process. Thus, the methods of claims 67 and 68 are *prima facie* obvious in view of the combined teachings of Myakishev, Piggee, Nolan, and Woolley.

Response to Amendment

11. The declaration under 37 CFR 1.132 filed on January 9, 2008 is sufficient to overcome the rejection of claims 23-25 and 39-41 under 35 U.S.C. 103(a) in view of the combined teachings of Myakishev, Piggee, and Weisner and the rejection of claims 67 and 68 under 35 U.S.C. 103(a) as being unpatentable in view of the combined teachings of Myakishev, Nolan, and Weisner. These rejections have been withdrawn. As noted by Dr. Slepnev in points 8 and 9 of the declaration, the teachings of Weisner cannot be incorporated into the method resulting from the combined teachings of Myakishev and Piggee.

However, the declaration under 37 CFR 1.132 filed on January 9, 2008 is insufficient to overcome the rejections of claims 18, 19, 21-35, 37-49, 62, 63, and 65-73 made under 35 U.S.C. 103(a) citing Myakishev as the primary reference, because: (i) the showing is not commensurate in scope with the claims, and (ii) the facts presented are not germane to the rejection at issue. In points 6 and 7 of the declaration Dr. Slepnev argues that the claimed method is directed to the simultaneous amplification and detection of multiple different polymorphic sites (specifically SNPs) in a sample, and that one of ordinary skill in the art would not have a reasonable expectation of success in combining the teachings of Myakishev, Piggee, and Nolan to obtain such a method. However, the claims do not require that the amplification is performed in a multiplexed fashion. As a result, Applicant's arguments regarding the ability of the method taught by Myakishev to be multiplexed are not germane to the rejection at issue, since the claimed method does not require this step. Also, since the claims do not require multiplexed amplification, the showing made in points 6 and 7 of the declaration is not commensurate in scope with the claimed invention.

Response to Arguments

12. As noted above, the rejection of claims 1, 2, 5, 10, 12, 18, 19, 22, 27, 34, 35, 38, and 43 under 35 U.S.C. 102(b) as being anticipated by Myakishev has been withdrawn in view of the amendment, and new grounds of rejection have been presented. Some of Applicant's arguments filed on January 9, 2008, regarding the Myakishev, Piggee, and Nolan references, remain pertinent to the new grounds of rejection. These arguments have been fully considered, but they were not persuasive.

Applicant first argues that Myakishev does not teach that "the identities of the nucleotides at a set of known polymorphic sites are interrogated in one reaction as set out in claims 18 and 34" (see pages 15-18 of the response). This argument was not persuasive, because the claims do not require that the method comprises multiplex amplification and simultaneous detection of the set of polymorphic sites. The claimed methods recite open, "comprising" language, and therefore, do not preclude conducting a plurality of separate amplification reactions to analyze each of the different polymorphic sites listed in Table 1 of Myakishev and pooling the resulting distinctly sized amplification products for detection by the capillary electrophoresis method of Piggee. The claimed methods also do not prohibit conducting a plurality of separate detection steps by capillary electrophoresis. Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

Applicant also argues that Myakishev does not teach a method "wherein said upstream amplification primers are selected such that each polymorphic site of said set of known polymorphic sites to be interrogated corresponds to a distinctly sized amplification product" (see

pages 16-17). This argument was not persuasive, because amplification of the nine polymorphic sites listed in Table 1 of Myakishve using the primer pairs also listed in Table 1 produces distinctly sized amplification products (see the table presented above on page 8 with regard to claims 18 and 34). Also, although Applicant correctly notes that Myakishev teaches that the size of the resulting amplification products is not critical, the fact remains that the amplification products produced by the method inherently have different (*i.e.* distinct) sizes, and therefore, they are suitable for analysis via a size-based separation method, such as the capillary electrophoresis method taught by Piggee.

Regarding the combination of the Myakishev and Piggee references, Applicant first argues that the secondary reference (Piggee) does not remedy the deficiency in the primary reference (Myakishev) with respect to multiplex amplification (see page 19). This argument was not persuasive, because as discussed above, the claims do not require simultaneous multiplexed detection or multiplexed amplification. Also, regarding Applicant's arguments related to reasonable expectation of success, it is noted that analysis of each polymorphic site only requires two distinguishable labels. That is, different polymorphic sites within the set of polymorphic sites to be interrogated may have the same label provided that the size and/or charge of the resulting amplification products differs. As noted above, the primers used in the method of Myakishev produce distinctly sized amplification products, and therefore, an ordinary artisan interested in analyzing the amplification products generated by the method of Myakishev using the capillary electrophoresis method of Piggee, could continue to use two-color fluorescence analysis, when the method is conducted using downstream primers having two different fluorescent labels (*i.e.* the method of all of the pending claims except claims 28, 44, 62, and 73).

In the method of claims 28, 44, 62, and 73, four labels could be used, as suggested by Piggee and Nolan, with a reasonable expectation of success. Furthermore, as discussed above, the claimed methods do not require simultaneous amplification or detection steps, and therefore, two-color or four-color fluorescence analysis could be used with a reasonable expectation of success to practice the methods resulting from the combined teachings of Myakishev and Piggee.

Applicant further argues that the use of different length primers as suggested by Piggee will not necessarily result in different length amplification products. This is correct. However, as discussed above, the primers used in the amplification method taught by Myakishev result in different length (*i.e.* distinct) amplification products (see the table on page 8 of the Office Action). The teachings of Piggee regarding the primer length are cited as one of many ways to obtain distinct amplification reaction products for use in a method resulting from the combined teachings of Myakishev and Piggee.

Applicant also argues that the combined teachings of Myakishev and Piggee do not result in all of the limitations of claims 28, 44, and 73 (see page 20). This argument was not persuasive, because the rejection of these claims further cites the Nolan reference. Also, as discussed above, the claims do not require multiplexed amplification, and therefore, the claims do not require that the four subsets of distinguishably labeled downstream primers recited in claims 28, 44, and 73 amplify more than one polymorphic site in the amplification step. In other words, the claims do not preclude a plurality of different amplification steps, each conducted with a different set of distinguishably labeled downstream amplification primers, as suggested by the combined teachings of Myakishev, Piggee, and Nolan.

Regarding the combination of the Myakishev, Piggee, and Nolan references, Applicant first argues that the teachings of the secondary references (Piggee, Nolan) do not remedy the deficiencies in the primary reference (Myakishev) (page 26). This argument was not persuasive for the reasons set forth above. Applicant also argues that teachings of Nolan are not applicable to the claimed methods, since the method of Myakishev is not readily amenable to multiplexing and refers to the attached Slepnev declaration for additional discussion (see pages 26-28). This argument was not persuasive, because as discussed in the previous section and above, the claimed methods do not require the degree of multiplexing described by Applicant. Briefly, the claims require multiplexed amplification of a population primer extension products having four different tags, each tag corresponding to a different nucleotide (A, G, T, or C) at the polymorphic site using at least two upstream primers and at least eight downstream primers but doesn't require multiplexed amplification in step VII. The claims also do not require simultaneous detection of the resulting amplification products. Therefore, Applicant's arguments regarding reasonable expectation of success and multiplexing are not persuasive.

Finally, Applicant argues that the method recited in claims 34 and 62 requires the upstream and the downstream primers to posses a tag, whereas the cited references only teach a tag on the downstream primers (see pages 29-30). This argument was not persuasive, because the specification does not limit a "tag sequence" to non-complementary sequences (see the definition of tag sequences on pages 17-18 of the specification). Therefore, as noted above, any portion of the reverse primer taught by Myakishev is a "tag sequence". It is noted that this interpretation is not inconsistent with the specification as asserted by Applicant, because the definition of a "tag sequence" recited on pages 17-18 of the specification only recites a non-

complementary "tag sequence" as a preferred embodiment. Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

Conclusion

13. No claims are currently allowable.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Chen (US 2003/0096277 A1; cited on an IDS) teaches a genotyping method that comprises allele-specific PCR with tagged primers followed by further amplification with labeled primers (see Figures 1-2 and pages 2-5). Huang et al. (US 6,287,778 B1) teaches a genotyping method that utilizes tagged primers (see Figures 1-2 and columns 1-3 & 5).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ANGELA BERTAGNA whose telephone number is (571)272-8291. The examiner can normally be reached on M-F, 9- 5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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